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I. Do Guinea Pigs Injected with Dead Bacterial Substance.

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Some Observations on Bacterial Hypersensitiveness.

I. In Guinea Pigs Injected with Dead Bacterial Substance.

Attention was first directed to bacterial hypersensitiveness by the phenomenon of anaphylaxis to non-bacterial antigens, such as horse serum. Rosenau and Anderson (1) claim that hypersusceptibility can readily be induced in guinea pigs with protein extracts obtained from the bacterial cell. They succeeded in sensitizing guinea pigs by single subcutaneous injections of extracts of colon, subtilis, anthrax, tubercle, and typhoid bacilli. On reinjection, usually 21 to 35 days later, with a rather large quantity (5 to 10 c.c.) of the extract, subcutaneously or intraperitoneally, they elicited symptoms which they characterized as slight, mild, marked, or severe. There were no lethal reactions, nor were convulsions noted in any case. Kraus and Doerr (2) produced anaphylactic shock by injecting, intravenously, suspensions of the bacteria of typhoid, dysentery or cholera into guinea pigs which had received, 20 to 25 days previously, a smaller amount of the corresponding organism subcutaneously. They recorded severe symptoms, with frequently exitus. Weil and Braun (3) were unable to produce hypersensitiveness to bacteria. Later Holobut by giving a series of sensitizing injections was able to obtain more regularly successful results.

Bacterial sensitization is by no means as readily effected as is sensitization to blood sera; this difference has been attributed (4) to the comparatively small amount of protein contained in even thick bacterial emulsions. It seems likely, however, that differences in the physicochemical state of the anaphylactogenic substance are to a considerable degree responsible.

The strict specificity of bacterial anaphylaxis was denied by Delarge (5), who claimed that animals sensitized with typhoid bacilli reacted (in vitro) when injected also paratyphoid A, paratyphoid B, or colon bacilli. Doerr and Pantzhoff (6) likewise held the specificity to be only relative. The majority of workers, however, consider the reaction specific within the same limits as other serum reactions. Kraus and Doerr (2) found that animals sensitized to typhoid bacilli reacted to typhoid, but not to paratyphoid or cholera; similarly those sensitized with cholera vibrios did not react to typhoid bacilli.

Anaphylaxis, within the strict meaning of the term now generally accepted, namely a state of hypersensitiveness that is due to the presence, in certain tissues, of specific antibodies, the symptoms of anaphylaxis being caused by the reunion of these antibodies with the respective antigen in those tissues, agrees in specificity, accordingly to Doerr (7), with that exhibited by the other immunological reactions, such as those of specific precipitation and complement - fixation. To quote Doerr: "All of the attempts to refer the anaphylactogenic function and the other known antigenic functions to different elements in the same protein material have failed; indeed, there is strong direct evidence of the identity of the anaphylactogenic and the precipitinogenic elements in such material."

As to the relationship of the local (cutaneous) manifestations of sensitiveness to the general systemic hypersensitiveness definitely known to be anaphylactic, there has been until comparatively recently much confusion. Thus as late as 1912 it was, according to Volmer (8), generally agreed that cutaneous hypersensitiveness and ana-

anaphylactic shock are identical, and the former was considered accountable as a delicate index of hypersensitiveness to a certain foreign protein.

Baldwin, however, showed that guinea pigs which had been treated with tuberculoprotein often gave evidence of anaphylactic sensitization, while sensitiveness resulted only from actual infection with living tubercle bacilli. These observations were confirmed and further extended by Krause. Fleischner, Hoover, and Shaw (9) later found this rule to apply to B. abortus bovinus and B. melitensis infections in guinea pigs.

Besredka (10) then distinguished clearly between two different types of skin reactions in the guinea pig. The first, which he designates the "immediate" reaction, is a wheal which appears within 15 minutes after intradermal injection of the antigen, lasts from 1/2 hour to 2 hours, then fades without leaving any profound injury of the tissues. He found that this reaction occurred generally, though not always, in guinea pigs sensitized to horse serum. The second type, or "delayed" reaction, first manifests itself in 4 or more hours as a swelling which in the course of 12 to 24 hours develops into an edematous area, often with central necrosis, and, occasionally, hemorrhage. This reaction may not reach its highest development until about 48 hours after the injection. The intradermal tuberculin test is a classical example of this reaction, which, as stated above, was not been found to occur except with actual infection.

[illegible]erimental.

One virulent and one avirulent strain of C. diphtheriae were used as antigens. Park 8 "new", largely used in toxin production, served as the virulent organism; the avirulent one was No. [illegible], derived from a single-cell culture isolated in this laboratory by [illegible]. These were cultured in Smith broth, to which .1% glucose had been added just before inoculation. The cultures were incubated for 10 days. The bacteria were then sedimented out by centrifugalization. The supernatant bouillon was filtered through a Berkefeld "medium". The bacterial mass was washed 3 times with .9% NaCl solution, and resuspended in approximately 4 times its volume of salt solution. This suspension was then allowed to stand for two days at room temperature, with an occasional shaking, and was then heated at 60° C. for 40 minutes.

Each animal in the two groups of guinea pigs treated with the bacterial antigen 1660 received a series of intraperitoneal injections of the bacterial suspension. At varying intervals thereafter several animals in each group were tested for cutaneous reactiveness or for general sensitization. The skin reactiveness was tested for by injecting intracutaneously .1 c.c. of each of the two bacterial suspensions, using a dilution of 1:2, and .1 c.c. of each of the corresponding broth filtrates, undiluted. The sites of injection were observed during the first 1/2 - 1 hour, and after 24 and 48 hours. Normal animals were similarly injected each time, as controls.

General reactiveness was tested for by injections into the peritoneal cavity, jugular vein, or heart, as specified in the protocols below. In the interpretation of slight anaphylactic reactions, the subjective element of course plays a considerable role, and enters in as a factor of error. Shock may be produced by the injection even of nor-

eal animals of large quantities of protein material, as 5 c.c. of bouillon into a small guinea pig. The comparatively small amounts of bacterial suspension used in the second series were never observed to produce any even suggestive reaction in the controls. A well defined anaphylactic shock is quite characteristic and cannot be confused with non-specific shock. Unless the reaction was considered entirely characteristic, it was sought to err on the side of "conservatism" and regard it as non-anaphylactic.

In reading the reactions, rapid and fast trot seemed workable, because of the wide variation in combination of symptoms in different individuals. In general, however, any typical reaction progressing to severe muscular twitchings or to convulsions, and with pronounced dyspnea, was considered severe; a reaction with marked dyspnea but no convulsions, moderate. The slight and mild reactions include those characterized by agitation, ruffling of the fur, sneezing or coughing, scratching of nose with the paws, pawing of muzzle to perineum, and discharge of feces and urine, with slight disturbance of respiratory rate or rhythm, followed by a complete return to normal within 10 to 30 minutes, or by a somnolence lasting an hour or more. Some one or other of these elements may be missing in individual cases.

Series A.

Guinea Pigs nos. 1, 2, 4, 5, and 6.

Sensitizing injections intraperitoneally of virulent bacillus 1000. Each dose = 0.06 mg. moist bacterial mass, in 2 - 3 c.c. salt sol. First injection on 12-7-22. Subsequent injections 2, 5, 7, 9, and 12 days after first.

Test [illegible].

[illegible] after first Injection:

G. P. [illegible] [illegible] gm.

Days.
[illegible] - [illegible] c.c. 1:[illegible] dilution of bacterial suspension 1660 intra-
peritoneally - [illegible] symptoms.

[illegible] - Intracutaneous tests- [illegible].

[illegible] - [illegible] c.c. Filtrate 1950 i.p.- No symptoms.

G. P. [illegible] [illegible] gm.

Days.
[illegible] - [illegible] c.c. 1:6 dil. susp. 1[illegible]60- i.p. No symptoms.

[illegible] - [illegible] c.c. Filtrate Park [illegible] New, intracardiac- slight
reaction, questionable. Recovery with 30 minutes.

G. P. [illegible]4. [illegible] gm.

Days.
[illegible]6 - Intracutaneous tests- Negative.

[illegible] - " " "

[illegible] - " " "

[illegible] - [illegible] c.c. Filtrate Park & New intracardiac- Moderately
sever symptoms, not characteristic.

G. P. [illegible]5. 425 gm.

Days.
70 - 2.5 c.c. Smith broth intracardiac- Mild shock, symptoms
atypical.

G. P. [illegible]. [illegible] gm.

Days.
26 - [illegible] c.c. 1:[illegible] dil. suspension bacilli 1660 intraperitoneal-
No symptoms.

62 - Intracutaneous tests- Negative.

83 - 1.5 c.c. suspension bacilli Park & New intracardiac-
Mild symptoms, doubtful.

G. P. 3. 650 gm. (Normal animal from stock)

3 c.c. saline broth intravenous- Mild, recovered shock, atypical.

Series C.

Guinea pigs Nos. 13, 14, 15, 16, 17, 18.

Sensitizing injections, intraperitoneal, of avirulent bac. 1660. Each dose consisted of 1 c.c. 10% original suspension, representing 0.2 gm. of moist bacterial mass, made up to 2 c.c. with salt sol. First injection I-22-25. Subsequent injections 2, 4, 6, 8, and 10 days after first.

Test Injections.

Days after First Injection:

G. P. #13. 190 gm.

Days.

16 - 0.3 gm. bac. 1660 in 3 c.c. sol., intraperitoneal- No symptoms.

24 - Intracutaneous tests- Negative.

26 - 0.06 gm. bact. Park & New in 1 c.c. sol., intracardiac- Marked symptoms. Moderate grade anaphylactic shock. Recovery.

G. P. #14. 280 gm.

Days.

24 - Intracutaneous tests- Negative.

30 - 0.15 gm. bact. 1660 in .75 c.c. sol., intracardiac- Moderate grade anaphylactic shock. Recovery.

G. P. #15. 270 gm.

Days.

30 - Intracutaneous tests- Immediate reaction - Doubtful.
Delayed " - Negative.

36 - Killed.

G. J. #16. 220 gm.

Days.
74 - 0.4 gm. bact. 1660 in 4 c.c. sol., intraperitoneal-
slight reaction of doubtful nature.

76 - 0.1 gm. bact. 1660 in 1.5 c.c. sol. intramuscular- Mild
symptoms. Reaction typical characteristic tissue not
recovered.

G. J. 17. 200 gm.

Days.
10 - Intracutaneous test- [illegible] positive.

60 - 1/4 Dose agar slant B.coli in 1 c.c. intravenously-
No symptoms within 15 min.

G. F. #18. 190 gm.

Days.
30 - 0.4 gm. bact. 1660 in 4 c.c. sol. intraperitoneal-
Reaction suggestive, but not clear-cut.

74 - Intracutaneous test- Immediate reaction - positive.
Delayed " - negative.

76 - 0.2 gm. bact. 1660 in 1 c.c. sol. intracardiac-
Severe symptoms. Reaction typical.
Killed after 1 hr. Tissue enthusiastically intended.

G. P. Yale. (Normal animals from stock)

Received amounts corresponding to above test injections, and by same routes. No symptoms.

The results may be summarized as follows:

What was regarded as a positive "immediate" skin reaction was observed in one instance. This animal proved definitely anaphylactic. A doubtful "immediate" skin reaction obtained in one case; in this it was not possible to test the general sensitiveness.

The "delayed" skin reaction, the index of true cutaneous sensitiveness, did not develop in any of the animals tested.

The animals in the second series were rendered definitely anaphylactic to the bacterial antigen.

The animals in the first series, however, did not show definite anaphylactic sensitization. This difference may have been due to the difference in the material used for test injections, or to the smaller sensitizing doses, or to the lesser size or greater age of the animals.

An animal sensitized with avirulent diphtheria bacilli was rendered anaphylactic to a virulent strain.

Conclusions:

Injection into guinea pigs of dead bacterial substance is capable of inducing the development of anaphylaxis, but a cutaneous hypersensitiveness, as indicated by the delayed type of reaction to intracutaneous injection of antigen, is not produced by this procedure. This is entirely in accord with several observations previously made with other organisms by a number of workers, as mentioned above.

The development of the immediate type of skin reaction is not a regular occurrence. Its presence in the one case which reacted rather severely to a test injection of the guinea pig's skin indicates that it is present only in cases of marked general hypersensitiveness.

The development of anaphylaxis to a virulent strain of B. diphtheriae by sensitization with an avirulent strain is interesting in view of the fact that these organisms were shown by Powell to fall into different groups with respect to specific agglutinin production in the rabbit. It is, to be sure, extremely hazardous to attempt deductions with any degree of assurance upon the basis of a single observation; nor would even any number of similar observations suffice, obviously, without at least a parallel determination of specific agglutinins in the animals used for tests, or other direct evidence, as passive transfer from rabbits to guinea pigs. Assuming, however, that such experiments would confirm what appears to obtain here, namely a lack of parallelism between the production of agglutinins and of anaphylaxis; the result would be at variance with the current view as expressed by Coca, which is that the anaphylactogenic and the other antigenic elements in the same proteid are identical.

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